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L. H. Thompson

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Larry H. Thompson

Biosciences Directorate, Biomedical Division, Lawrence Livermore National Laboratory, P.O. Box 808, Livermore, California, 94551-0808, USA. e-mail: Thompson14@llnl.gov

How the Fanconi anaemia (FA) chromosome stability pathway functions to cope with interstrand crosslinks and other DNA lesions has been elusive, even after FANCD1 proved to be BRCA2, a partner of Rad51 in homologous recombination. The identification and characterization of two new Fanconi proteins having helicase motifs, FANCM and FANCJ/BRIP1/BACH1, implicates the FANC nuclear core complex as a participant in recognizing or processing damaged DNA, and the BRIP1 helicase as acting independently of this complex.

Fanconi anaemia is a chromosome instability disorder characterized by diverse congenital abnormalities, retarded growth, early predisposition to cancer, and bone marrow failure. The hallmark of FA cells is their high sensitivity to chromosomal breakage and killing caused by exposure to DNA interstrand crosslinking agents (e.g. mitomycin C, cisplatin). This property has long suggested that a major biochemical defect underlying FA involves defective repair of these uniquely toxic lesions. The search for the mechanistic basis of FA proteins at the chromosomal level has been a protracted endeavor, with the first cloning of an FA gene (*FANCC*) in 1992 when there were just four known complementation groups[1]. Since then, the count has climbed to 11 groups representing distinct genes (*FANCA/B/C/D1/D2/E/F/G/I/J/L*)[2]. Remarkably, only one of the encoded proteins, FANCD1/BRCA2[3], is implicated directly in DNA repair (via homologous recombination, HR), and its functional relationship to other FANC proteins has been obscure. Now, two articles in this issue reveal the identity of FANCJ to be a previously discovered DNA unwinding enzyme (helicase) designated BACH1/BRIP1[4]. Studies by Levitus *et al*.[5] on page XXX, and by Levran *et al*.[6] on page XXX, localized *FANCJ* to a region on chromosome 17q22, pursued *BRIP1* as a likely candidate, and identified biallelic *BRIP1* mutations in FA patients. Extending this finding further in chicken DT40 cells, Bridge *et al*.[7] report on page XXX the characterization of a *brip1* knockout mutant; they discover that its phenotype is essentially identical to that of a *fancc* mutant. Adding further excitement to the

helicase story, Meetei *et al*.[8] (page XXX) used mass spectrometry to identify yet another new FA protein, FANCM/FAAP250, as a member of the FANC core complex that has homology with both helicases and endonucleases, in particular the dual-domain archaeal Hef protein. Concurrently, Mosedale *et al*. [9] provide insightful characterization of *hef/fancm* partial deletion mutants of DT40 cells.

Linkage to DNA repair

We may now hold a critical missing link within the FANC pathway, i.e. two new enzymes that interact with DNA. BRIP1/BACH1 was first identified as an interactor with the BRCA1 tumour suppressor[4]. BRIP1 was shown to be a 5′ to 3′ helicase, and germline mutations affecting the helicase function were detected in two early-onset breast cancer patients[10]. BRIP1, like BRCA1, was implicated in repairing double-strand breaks (DSBs) produced by ionizing radiation[4]. However, the normal X-ray sensitivity of the *brip1* DT40 mutant argues against a role in such DSB repair[7]. Moreover, a truncated human *BRIP1* cDNA lacking the C-terminal BRCA1-interacting region, fully corrected *brip1* cells for sensitivity to cisplatin whereas a helicase-inactivating point mutation (Walker A-box K52R substitution) did not, implying that BRIP1's helicase activity is essential for resistance to crosslinks. A *brip1 fancc* double mutant closely resembles the single mutants, further suggesting that BRIP1 acts within the same pathway as the FA core complex proteins.

Very recently, characterization of the substrate specificity of purified recombinant BRIP1 showed that it preferentially binds and unwinds forked duplexes[11]. With model substrates that mimic the HR structure known as a D-loop, BRIP1 can also release the invading strand, even when it lacks a single-stranded tail. Unlike BLM helicase, which is curiously associated with FANC core complex proteins[12], BRIP1 fails to unwind synthetic 4-way structures that model the classical Holliday junction[11].

FANCM/FAAP250/Hef contains two potential enzymatic domains[8]. One is homologous to a superfamily of DEAH-box helicases or DNA-stimulated ATPases, and the other homologous to the endonuclease domain of XPF/ERCC4. However, the C-terminal endonuclease domain of FANCM has diverged from XPF and other nucleases to the extent it is expected to be nonfunctional. In contrast, FANCM's helicase domains are highly conserved compared with Hef helicases in archaea and the yeast MPH1 helicase. Although FANCM actually shows no helicase

activity, it is able to displace the third strand of a triple-helix structure in the presence of ATP, suggesting the ability to move along DNA (translocase activity).

Two sibling FA patients from an undefined complementation group have mutations in both alleles of FANCM[8]. Attempts to complement *fancm* mutant cells with *FANCM* cDNA were unsuccessful, perhaps because of inappropriate expression from viral promoters. Each protein in the FA core complex (FANCA/B/C/E/F/G/L) is essential for monoubiquitination of FANCD2, a critical reaction of unknown biochemical mechanism in the FANC DNA-damage response pathway[13]. FA-M cells also lack FANCD2 monoubiquitination and have impaired nuclear localization and defective assembly of other core complex components[8].

Using chicken cells, Mosedale *et al*. [9] provide key evidence that FANCM/Hef is essential for mediating binding of the core complex to crosslinked DNA within chromatin. Not only do they find that a *hef* mutant disrupted in the helicase domain is essentially epistatic with *fancc* for crosslink sensitivity in a double mutant, they show that the nuclease domain of Hef is largely dispensable for resistance to DNA crosslinking.

Assembling the FAscinating puzzle

How does the discovery of these two helicase-motif proteins help us understand the repair of DNA crosslinks? A recent mechanistic model proposed that the role of FANCD2 monoubiquitination is to stabilize structurally blocked and broken replication forks, and thereby promote both translesion synthesis (TLS) and HR[14]. In the context of this model, an outline of events that may occur during crosslink repair is depicted in Figure 1. Crosslinks are probably recognized when they block DNA replication forks. Crosslink "unhooking" requires incision on both sides of an adducted base, causing breakage of one parental strand at the replication fork (Fig. 1, step B). The ERCC1-XPF endonuclease likely produces one or both incisions[15]; mutations in either protein are known to confer high crosslink sensitivity. One can speculate that FANCJ/BRIP1 helicase activity might help create the incision substrate by unwinding the parental strands next to the crosslink and/or unwind the short adducted oligonucleotide after incision. Restoration of the broken fork requires HR (steps C-E), which involves FANCD1/BRCA2 and other proteins that enable Rad51 to perform strand transfer. After recombination, another hypothetical role for BRIP1 might be to assist in restarting replication.

Much more FANC protein complexity remains to be elucidated. One reported complementation group (FA-I) has not been cloned[2]. FANCI is also required for FANCD2 monoubiquitination but, uniquely, does not appear to be a component of the nuclear core complex[2]. Clearly, many additional protein interactions and temporal sequences of events must be established to understand crosslink repair. A central question that needs resolution is whether the spontaneous chromosomal breakage of FA cells is caused by endogenous crosslinks or simply other lesions arising from oxidative base damage.

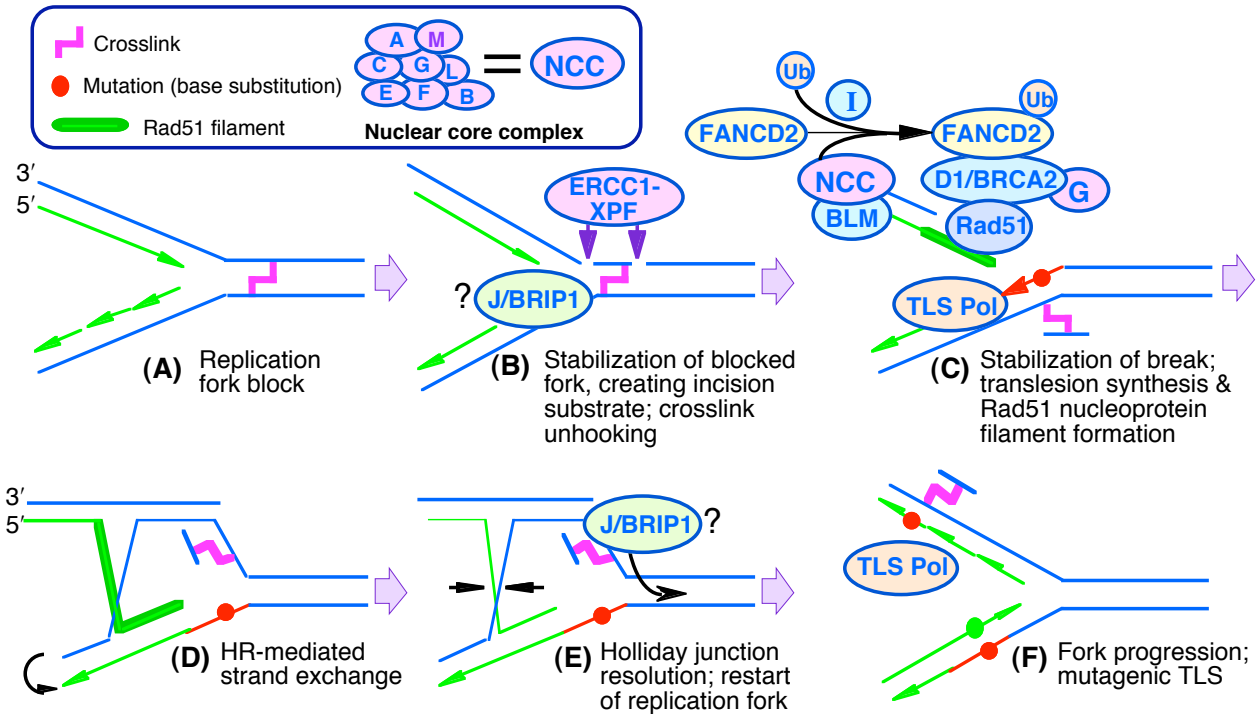

FIGURE LEGEND

Figure 1 Speculative heuristic model of FA proteins in repairing interstrand crosslinks during DNA replication. Upon replication fork arrest/breakage, FANCD2 becomes monoubiquitinated, a key event required for resistance to crosslinks. This modification is proposed to promote both translesion synthesis (TLS) at blocking adducts and HR to restart broken forks, by architecturally stabilizing intermediates[14].

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